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(54) Blood cell separation.

(57) Hydroxyalkyl celluloses are useful as sedimenting agents in the non-destructive separation of red and white blood cells. Intact WBC recovery is higher than with conventional methods. The white blood cells may be used in interferon production.

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Description

BLOOD CELL SEPARATION

BACKGROUND OF THE INVENTION

This invention relates to the separation of leukocytes and erythrocytes by sedimentation at normal gravity in the presence of a hydroxyalkylcellulose. The leukocytes may be used in interferon production.

Information Disclosure Statement

Hydroxyethyl starch is routinely used as a sedimenting agent. See Lionetti, US 4,004,975; Pestka, US 4,289,690; Djerassi, US 4,111,199; Chadha, US 4,485,038; and Van Oss, et al., Immunol. Commun., 10(6):549-55 (1981). Treatment with HES results in recovery of about 68% of the original pool of WBCs, while the present method recovers 80%. Ammonium chloride lysis yields 90% of the WBCs, but is undesirable in view of the wastage of the RBCs.

Other agents used for this purpose are Dextran (glucose polymer), Ficoll (sucrose polymer), PVP, fenugreek seed extract, and phytohemagglutinins. Lichtenstein, US 3,709,791; Chany, US 3,560,611; Ferrante, US 4,255,256; Guirgis, US 4,152,208; Furuta, US 4,409,106; Goore, US 3,800,035; Shepherd, US 3,594,276; Kirkham, US 3,635,798; Widmark, US 3,700,555.

Kanter, US 4,487.700, used a thixotropic barrier material of intermediate density to separate lymphocytes from erythrocytes and phagocytized leukocytes. Meyst, US 4,283,289 described a leukocyte filter. The use of NH_4Cl to lyse RBCs, while leaving most WBCs intact, is also known.

Hydroxyalkyl celluloses, and particularly hydroxyethyl cellulose, are used in pharmaceuticals and other compositions as a thickening and stabilizing agent. They have not, however, previously been used as a cell sedimenting agent.

SUMMARY OF THE INVENTION

This inverntion relates to the use of hydroxyalkyl celluloses, particularly hydroxymethyl cellulose (HMC), hydroxyethyl cellulose (HEC) hydroxypropyl cellulose, (HPC), and hydroxybutyl methyl cellulose (HBMC) as sedimenting agents in blood cell separation. By the method of this invention, about 80% of the oroginal number of leukocytes are recovered, intact, with over 95% in a viable state, from the upper phases, Moreover, the erythrocytes in the lower phases are also left intact.

Since the final concentration of HEC in the mixture is 0.05%, as compared to 3% HES in the conventional separation scheme, the harvested cells have relatively little HEC bound to them and may be washed off easily.

Unlike NH_4Cl lysis, the HEC technique does not destroy granulocytes (granular leukocytes) or erythrocytes. Morevoer, the WBCs recovered by this technique may be stored for at least one day without impairment of alpha interferon production after exposure to an inducer.

DETAILED DESCRIPTION OF THE INVENTION

Example 1

Buffy coats (American Red Cross) are pooled, mixed and sampled. Initial RBC and WBC counts are determined using the Cell Dyn 400 cell counter (Sequoia International). A sedimenting agent is prepared by making a solution of 0.1% of HEC (w/v) and 0.9% of NaCl (w/v) in deionized water, stirring for at least one half hour until the HEC goes into solution. The sedimenting agent is slowly mixed with the buffy coat pool for at least one minute, poured into a separatory funnel, or other suitable vessel and allowed to settle for 1 1/2 - 3 hrs. at room temperature or colder. The final concentration of HEC in the mixture is preferably 0.05% (w/v). However, HEC may be present in a final concentration of from 0.025% to 0.5%. After completion of the separation the bottom RBC layer is drained or the top WBC layer is aspirated. The bottom layer may be residimented as previously described with fresh sedimenting agent to capture more WBCs.

The collected layers were tested for RBC count, WBC count, WBC/RBC, and WBC viability (by Trypan blue exclusion test). The results were as follows:

Sample	Volume	RBC*10^{12}	WBC*10^{10}	WBC/RBC	Yield
Experiment 1476-19 (0.1% HEC, 0.9% NaCl stock solution)					
pool	600ml	3.25	4.00	0.01	100%
top layer	720ml	0.20	3.37	0.17	84%
bottom	500ml	2.73	0.34	0.001	9%
top layer (after wash)	160ml	-	3.12	-	78%
Experiment 1476-7 (0.1% HEC, 0.9% NaCl stock solution)					
pool	1000ml	5.88	4.66	0.01	100%
top layer	830ml	0.11	3.72	0.34	80%
bottom	1190ml	5.45	1.09	0.002	23%
top layer (after wash)	130ml	0.07	3.58	0.51	77%

Centrifugation may be employed to wash the harvested cells. Alternatively, the cells may be cleaned by tangential flow filtration or other conventional methods.

The WBC layer was spun at 1000 x g for 7 min. The supernatant was discarded and the pellet was resuspended in leukocyte medium at 37 degrees C. and a WBC count was performed. WBCs were added to a 2L volume at MEM medium, human serum, and alpha interferon primer at a concentration of 1x10°7 WBCs/ml. Induction took place in 6L round bottom flasks located in water baths maintained at 37°C. After an initial incubation period of 3 hours, Sendai virus was added to the flasks in order to induce alpha interferon production. The induction period was typically 18 hours. Alpha interferon was then harvested by centrifugation at 4000g's for 20 minutes. Samples of the supernatant were submitted for CPE (cytopathic effect) assay.

The CPE assay is based on the ability of alpha interferon to protect certain cells against certain viruses. In the assay used, Hep 2 Cells were grown to confluence in 96 well microtiter plates. Serial dilutions of the alpha interferon were added to the sample wells and incubated with the cells (37 C, 5%CO_2) for about 20 hours. Next, VSV virus was added to the wells to infect unprotected cells. After an incubation period sufficiently long enough to achieve 100% cell death in the control wells (i.e., only cells and virus present in these wells), all wells were stained with gentian stain. Intact cells appear purple due to the membrane picking up the stain. The well number (i.e. the dilution) at which 50% of the cells had been protected is called the endpoint. The endpoint was then used to correlate the titer of the sample (in units of interferon/ml.) to standards from the NIH.

When a stock solution of 0.05% HEC was used for cell separation, a very slow separation occurred. That is, when compared with separations using 0.1 to 0.2% HEC stock solutions, the 0.05% HEC separation was not as far along as the latter two in a comparable time period. Also, the 0.05% separation seemed to have more RBC contamination in the middle layer (as evidence by a pink band rather than the white one seen in each of the other separations).

A stock solution of 0.5% HEC did not give a good separation - only two layers formed and more WBCs were recovered from the bottom layer than from the top layer. Also, the "purity" of the top layer, as expressed in terms of WBC/RBC, was less than 1/2 of that seen in the combined two upper layers of the 0.1% HEC separation (i.e. 0.14 compared to 0.30). Concentrations of 0.7 and 1.0% HEC were even less effective.

Over a series of experiments, alpha interferon titers of 20-50,000 Units/ml were obtained.

A cytospin smear of HEC-collected WBCs was prepared. The cells were treated with MAY-GRUNWALD-GIEMSA stain, and inspected microscopically. A cell differential study showed the leukocyte subpopulations to have essentially the same distribution as in the original blood pool. The morphology of the leukocytes was normal.

Example 2

A 0.1% solution of hydroxypropyl cellulose (300,000 m.w.; Aldrich Chemical) was made as previously described for HEC. The 0.1% HPC/0.9% NaCl solution was mixed with an equal volume of buffy coat pool. In two separate experiments, 1476-19C and 1476-21, good separation between WBCs and RBCs was observed. However, since neither separation had been done in a separatory funnel it was difficult to effectively collect the top layer without contaminating it from RBCs from the bottom layer.

The same type of experiments were done with hydroxybutyl methyl cellulose (HBMC). Using the same 0.1% concentration of polymer with 0.9% NaCl, the separation appeared to occur faster and looked better than a concurrently run HEC separation.

Modifications

WBC yield may be increased by a number of means. First, the sedimentation may be carried out in a device facilitating RBC/WBC separation, such as a device having a constriction at the expected location of the RBC/WBC interface, or one having draining or aspirating means designed to minimize agitation of the interface. Second, a thixotropic agent may be added whose specific gravity is such that it will collect into a barrier structure separating the RBC and WBC layers. Third, the extraction step may be repeated as desired with fresh sedimenting agent.

Besides HEC and HPC, other hydroxyalkyl celluloses should be used as sedimenting agents.

HEC might be used, not only in unit gravity sedimentations, but also in RBC/WBC separation by centrifugation. HEC might also be used in the separation of cells from cell debris. The WBCs and RBCs provided by the present technique may be separated into WBC or RBC subtypes or fractionated to yield various WBC or RBC constituents.

Claims

1. A method of separating leukocyte and erythrocyte-containing blood or blood fraction into a first fraction enriched for leukocytes and a second fraction enriched for erythrocytes which comprises mixing the blood or blood fraction with a sedimenting agent comprising a hydroxyalkyl cellulose, permitting the mixture to settle and separating the mixture into said first and second fractions.

2. The method of claim 1 in which the settling occurs at unit gravity.

3. The method of claim 1 or 2 in which the final concentration of hydroxyalkyl cellulose in the mixture is 0.025% to 0.5%.

4.The method of any one of claims 1,2 or 3 in which the hydroxyalkyl cellulose is hydroxyethyl cellulose.

5. The method of any one of claims 1,2 or 3 in which the hydroxyalkyl cellulose is hydroxypropyl cellulose.

6. The method of any one of claims 1,2 or 3 in which the hydroxyalkyl cellulose is hydroxmethyl cellulose.

7. The method of any one of claims 1,2 or 3 in which the hydroxyalkyl cellulose is hydroxybutyl methyl cellulose.

8. The method of any preceding claim, in which over 80% of the original leukocytes are recovered in the leukocyte-enriched fraction.

9. The method of any preceding claim further comprising use of a thixotropic barrier material to separate the first and second fractions.

10. The method of any preceding claim, which does not comprise exposure of the cells to conditions which destroy erythrocytes or granulocytes.

11. A method of obtaining leukocyte cells suitable for interferon induction which comproses providing blood or a leukocyte-containing fraction of blood, mixing the blood with a sedimenting agent comprising a hydroxyalkyl cellulose, so as to leave an upper layer enriched in leukocytes, and recovering leukocytes from said upper layer which are suitable for interferon induction.

12. The method of claim 11 in which the leukocytes are suitable for interferon induction even after a day of storage.

13. The method of claim 11 or 12 in which the hydroxyalkyl cellulose is selected from the group consisiting of hydroxymethyl, hydroxyethyl, hydroxypropyl and hydroxybutyl methyl celluloses.

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EUROPEAN SEARCH REPORT

Application Number

EP 88 30 0325

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	CHEMICAL ABSTRACTS, vol. 86, no. 9, 28th February 1977, page 190, abstract no. 52440p, Columbus, Ohio, US; P.J. KERRY: "The isolation of ovine lymphocytes and granulocytes from whole blood using hydroxyethylcellulose", & RES. VET. SCI. 1976, 21(3), 356-7 * Abstract *	1,4	A 61 K 35/14
Y	IDEM	1-4,6, 10-13	
Y	EP-A-0 036 168 (MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN e.V.) * Page 9; page 14, last paragraph; claim 1 *	1-4,6, 10-13	
A	EP-A-0 028 842 (FERRANTE et al.)		
A,D	US-A-4 004 975 (F.J. LIONETTI et al.)		TECHNICAL FIELDS SEARCHED (Int. Cl.4)
A,D	US-A-3 560 611 (C. CHANY et al.)		A 61 K
A	CHEMICAL ABSTRACTS, vol. 69, no. 3, 15th July 1968, page 869, abstract no. 9263v, Columbus, Ohio, US; A. BOYUM: "Isolation of leukocytes from human blood. A twophase system for removal of red cells with methylcellulose as erythrocyte-aggregating agent" & SCAND. J. CLIN. LAB. INVEST., SUPPL. 1968, 21(97), 9-29		

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	19-04-1988	ALVAREZ Y ALVAREZ C.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A	CHEMICAL ABSTRACTS, vol. 69, no. 3, 15th July 1968, abstract no. 9264w, Columbus, Ohio, US; A. BOYUM: "Isolation of leukocytes from human blood. Further observations. Methylcellulose, dextran, and ficoll as erythrocyte-aggregating agents" & SCAND. J. CLIN. LAB. INVEST., SUPPL. 1968, 21(97), 31-50		

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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